ADC Case Study

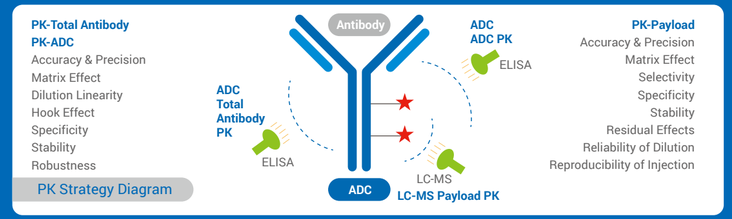
Background:

Consider the example of a marketed HER2 ADC drug. The antibody component of this drug specifically binds to human HER2. Once bound, the ADC enters the cell through internalization, releasing its payload within the cytoplasm, ultimately leading to cell death. Given the significant upregulation of HER2 protein in multiple cancerous tissues, ADC drugs targeting the HER2 receptor hold tremendous potential for treating breast cancer, gastric cancer, non-small cell lung cancer, and other cancers.

Assay Design:

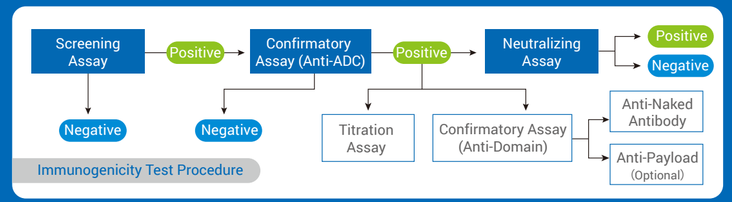
We offer customized services aligned with global regulatory guidance to support IND submissions. Our services include:

- **PK of Payload:** Determination of payload concentration in human serum/plasma using LCMS.
- **PK of Total Antibody:** Measurement of total ADC antibody concentration in human serum using ELISA.
- **PK of ADC:** Measurement of ADC concentration in human serum using ELISA.
- **ADA:** Detection of anti-ADC antibodies in human serum using the Electrochemiluminescence method.
- **NAb:** Detection of neutralizing antibodies against ADC in human serum using cell-based assays.

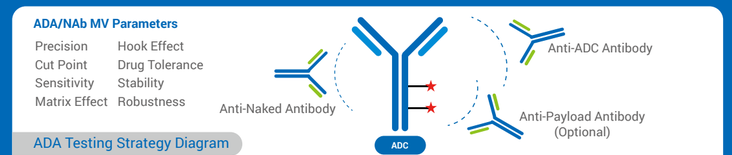


PK Strategy Diagram

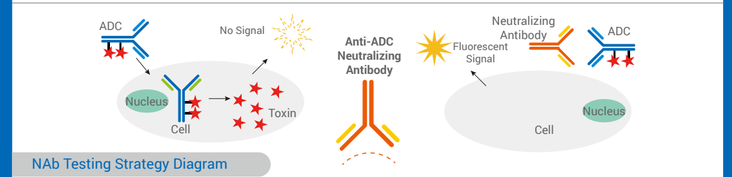
ADC Immunogenicity Testing Strategies



Immunogenicity Test Procedure



ADA Testing Strategy Diagram



NAb Testing Strategy Diagram

Our Advantages

Global Integrated Platform: We provide one-stop services to global sponsors, from preclinical to clinical stages, supported by multiple BAS sites worldwide.

Customized Services: Our sponsor-centric professional project management team delivers tailored services to meet your specific needs.

Experienced Team: Our core technical team members boast over 10 years of experience, with our management team having more than 20 years.

Statistical Services: Our immunogenicity cut-point determination offers expedited data delivery, ensuring you quickly get accurate, reliable results. Our services are grounded in scientific rigor, providing enhanced accuracy and dependable outcomes.

With more than 50 ADC projects under our belt, our expertise spans a broad spectrum of targets, including HER2, Muc1, CD70, ROR1, CD38, B7-H3, Trop-2, and more. Our extensive experience with payloads encompasses DM1, MMAE, MMAF, PBD, Exatecan, paf-AS269, Tubulysin analogs, and more.

Common Industry References:

1. Guidelines for Validation of Quantitative Analysis Methods for Biological Samples, Chinese Pharmacopoeia 2020 edition
2. Guiding Principles for Clinical Development of Anti-Tumor Antibody-Drug Conjugates, Center for Drug Evaluation of the National Medical Products Administration
3. Bioanalytical Method Validation and Study Sample Analysis M10, International Council for Harmonization
4. Bioanalytical Method Validation, U.S. Food and Drug Administration
5. Clinical Pharmacology Considerations for Antibody-Drug Conjugates, U.S. Food and Drug Administration
6. Immunogenicity Testing of Therapeutic Protein Products—Developing and Validating Assays for Anti-Drug Antibody Detection, U.S. Food and Drug Administration

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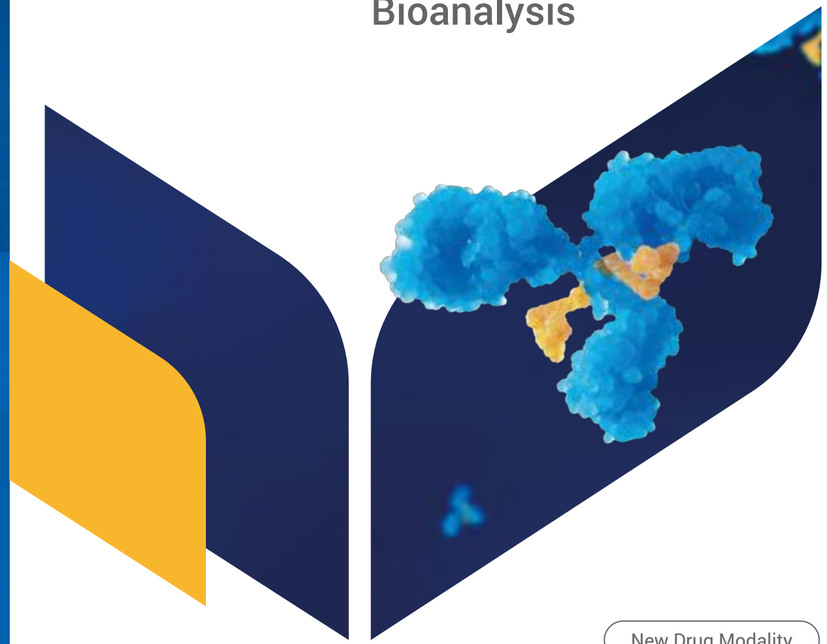
ADC

Antibody-Drug Conjugate Bioanalysis



New Drug Modality

Bioanalytical Services

Global Integrated Bioanalytical Platform

WuXi AppTec Bioanalytical Services operates globally and has leading integrated laboratories in China and the U.S. We adhere to GLP and GCP principles and strictly follow high industry regulatory standards. Our comprehensive platform, equipped with advanced instruments and staffed by experienced experts, provides professional one-stop services in preclinical/clinical mass spectrometry, immunochemistry, molecular/cellular, and central laboratory services to global clients.

Shanghai, China
Headquarters
Bioanalytical Services (Clinical)
Central Lab

Suzhou, China
Bioanalytical Services (Preclinical)
Antibody Service

New Jersey, USA
Bioanalytical Services (Preclinical/Clinical)
Central Lab

Chengdu, China
Bioanalytical Services (Preclinical)

Nantong, China
Bioanalytical Services (Preclinical)

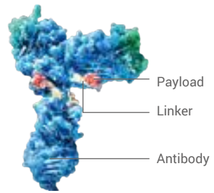
18+ Years of lab operations in China and the U.S.
24,300 m² lab space

5,000+ small molecule validated methods
2,800+ large molecule validated methods
2M+ samples analyzed per year

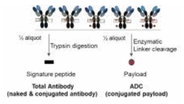
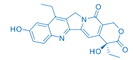
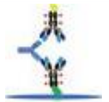
Global Regulatory Standards
Successfully passed global regulatory inspections, comply with GLP, GCP, CAP quality standards
200+ China NMPA
30+ International regulatory agencies (U.S. FDA, OECD, EMA, PMDA, CAP)

Antibody-Drug Conjugates (ADCs) are formed by linking biologically active small molecule drugs to antibodies using a chemical linker. This allows the antibody to serve as a carrier, delivering the small molecule drug directly to target cells.

WuXi AppTec's Bioanalytical Services (BAS) has supported more than 50 preclinical and more than 50 clinical studies across 10+ targets, showcasing our extensive expertise in method development. We offer a comprehensive and integrated ADC bioanalysis solution, aiding clients in quickly advancing their ADC drug development projects and overcoming various bioanalytical and clinical challenges. At any clinical research stage, our services provide thorough quantitative detection and analysis, successfully assisting sponsors in accelerating global IND, NDA, and BLA submissions.



Bioanalytical Solutions for ADC

Research Contents		Analyte	Platform	Assay Design
Pharmacokinetics/Toxicokinetics	PK/TK	ADC	ELISA/MSD	**Critical Reagents:** ▪ Anti-payload mAb ▪ Biotin labeled anti-CDR mAb or biotin labeled antigen ▪ SA-HRP 
		Total Antibody	ELISA/MSD	**Critical Reagents:** Antigen, anti-idiotype antibody or anti-CDR mAb or anti-human mAb 
		ADC/ Total Antibody	LCMS	**Critical Reagents:** ▪ Biotin labeled anti-ADC antibody ▪ SA-beads ▪ Linker lyase ▪ Isotope-labeled signature peptide (in-house synthesis) 
		Payload/ Linker-Payload	LCMS	Regular LC-MS assay 
Immunogenicity	ADA	Anti-ADC Antibody/ Anti-Naked Antibody/ Anti-Payload Antibody	MSD	Three confirmatory assays: ADC, naked antibody, and payload 
	NAb	Neutralizing Antibody	Cell-Based Assay (Recommended) or Non-Cell-Based Assay	The antibody binds to the target receptor, and through receptor-mediated internalization, the ADC is transferred into the cell, leading to the release of the payload and the exertion of its cytotoxic effect. On the other hand, neutralizing antibodies block the binding of the drug to the cell surface receptor, thereby inhibiting the cytotoxic effect of the drug. 
Pharmacodynamics/Biomarker	RO	Receptor Occupancy	FACS	The binding of the antibody to the target receptor is a crucial step in the action of ADC. Evaluating the receptor occupancy of the drug target in the appropriate cell populations and employing suitable detection strategies provide data support for studying drug efficacy and dosage. 
	Biomarker	Cytokines PD Marker	ELISA/MSD/ Luminex/ELLA/ Simoa/Gyros/ SMCxPRO, etc.	Biomarkers are objective indicators that can be measured and evaluated to assess normal biological processes, pathological processes, or responses to drug interventions. They are widely used in clinical practice as tools for diagnosis, prognosis, prediction, drug efficacy, safety, and monitoring. 

Challenges and Experience in ADC Bioanalysis

Challenges	
ADC	▪ DAR sensitivity ▪ The measured ADC concentration is higher than that of total antibody ▪ Some payload is pH sensitive and thereby leads to the release of toxins
Total Antibody	▪ DAR sensitivity ▪ The measured total antibody concentration is lower than that of ADC
Payload	▪ Release of toxins from ADCs during storage and analysis ▪ Conversion of payload into isomers during storage and analysis ▪ Low quantification limit
Anti-ADC Antibody Anti-Naked Antibody Anti-Payload Antibody	▪ Drug tolerance ▪ Anti-payload antibody: low immunogenicity, low specificity for positive controls, poor sensitivity
Neutralizing Antibody	▪ Drug tolerance ▪ Robustness ▪ Sensitivity
Receptor Occupancy	▪ Detection strategy ▪ Selection of target cell population ▪ Drug toxicity
Cytokines PD Marker	▪ Sensitivity ▪ Robustness ▪ Multiplex cytokine detection

Experience in ADC Bioanalysis

Target			Linker	Payload	
HER2	Cadherin	CD138	MMC	MMAE	AS269
Muc1	Claudin 18.2	CD48	MC	MMAF	Exatecan
CD70	CD46	CD73	MB	DM1/DM4	Eribulin
ROR1	CDH6	EGFR	VC-PAB	DXD	SN-38
CD38	IL-2	uPAR	GGFG	Tubulysin	
B7-H3	FR-α	CDH3	VA-PAB	MDC	
Trop-2	FGF3				